Endocytosis without clathrin coats

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Endocytosis is involved in an enormous variety of cellular processes. To date, most studies on endocytosis in mammalian cells have focused on pathways that start with uptake through clathrin-coated pits. Recently, new techniques and reagents have allowed a wider range of endocytic pathways to begin to be characterized. Various non-clathrin endocytic mechanisms have been identified, including uptake through caveolae, macropinosomes and via a separate constitutive pathway. Many markers for clathrin-independent endocytosis are found in detergent-resistant membrane fractions, or lipid rafts. We will discuss these emerging new findings and their implications for the nature of lipid rafts themselves, as well as for the potential roles of non-clathrin endocytic pathways in remodeling of the plasma membrane and in regulating the membrane composition of specific intracellular organelles.

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Endocytosis – the uptake of membrane proteins and lipids, extracellular ligands and soluble molecules from the cell surface – is a hallmark of all eukaryotic cells. A heterogeneous array of cellular processes involve endocytosis, including: nutrient uptake, synaptic vesicle recycling, regulation of cell-surface expression of signaling receptors, remodeling of the plasma membrane and the generation of cell polarity. To meet the differing requirements of such processes, endocytic pathways must possess considerable diversity in terms of regulation, specificity for different cargoes, and ultimate destination within cells. The best-studied endocytic pathway involves clathrin, which forms coated membrane invaginations on the plasma membrane that recruit cell-surface receptors and then, through a series of highly regulated steps, pinch off to form clathrin-coated vesicles[1,2]. Less well characterized, but equally important, are non-clathrin endocytic pathways. These pathways do not use known coat complexes for cargo recruitment and budding of transport intermediates. Instead, they might exploit lateral heterogeneity in plasma membrane lipid and protein composition to select cargo as well as scaffolding or structural components into dynamic membrane microdomains that bud into the cell.

Research focused on non-clathrin internalization pathways has blossomed recently for several reasons. First, the development of dominant-negative mutant proteins that specifically interfere with clathrin-mediated endocytosis has made it easy to determine whether a specific molecule is taken up by a non-clathrin pathway and to characterize the properties of this pathway in isolation from clathrin-mediated uptake[3,4]. Second, imaging of fluorescently tagged proteins and lipids in living cells has provided a means of visualizing movement of molecules through non-clathrin pathways and thereby to study where these pathways lead within cells and the conditions that affect them[5–7]. Finally, although there is little direct evidence as to what extent, and how, molecules are sorted into non-clathrin pathways, the possibility that some non-clathrin pathways depend on specialized lipid domains, or rafts, thought to be organizing platforms for membrane sorting and signaling events[8], has stimulated interest in understanding the roles rafts play in these uptake pathways.

Despite the growing list of ligands and receptors that utilize clathrin-independent uptake pathways (Table 1), we still know very little about the molecular mechanisms that underlie uptake by these pathways or exactly where these pathways lead inside the cell. This 'Opinion' article summarizes the emerging properties of different non-clathrin pathways, discusses their regulation and potential roles within cells and frames questions for future work in this area.

Diversity and roles of clathrin-independent pathways

A variety of endocytic pathways that do not utilize clathrin are responsible for taking up either large particles or small solutes, together with membrane, into cells. These pathways include phagocytosis, caveolae-mediated uptake, macropinocytosis and constitutive non-clathrin uptake. Phagocytosis is the process whereby large particles are internalized by cells. Uptake is typically triggered by binding of the particle to cell-surface receptors capable of transducing a phagocytic stimulus. This results in localized exocytosis at the site of particle attachment and subsequent pseudopod extension that wraps around and engulfs the bound particle into a cytoplasmic phagosome[9]. Phagocytosis is usually restricted to macrophages and other phagocytes that specialize in uptake and digestion of large particles.

Other distinct non-clathrin pathways underlie the uptake of smaller cargoes. They utilize either caveolae, macropinosomes or a little-understood constitutive process of plasma membrane internalization. A diverse array of molecular machinery is involved, including caveolin, ARF6, dynamin, ankyrin/spectrin and actin. Although there are few data on the organization of markers for non-clathrin pathways within the plasma membrane, it is striking that, at least where appropriate assays have been carried out, these markers have all been found to be present in lipid rafts (Table 1). Lipid rafts are thought to be dynamic, detergent-resistant regions of the plasma membrane enriched in cholesterol, glycosphingolipids, glycosylphosphatidylinositol (GPI)-anchored proteins and some membrane proteins[8,10]. By contrast, typical markers for clathrin-mediated endocytosis, such as the transferrin receptor and the

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Table 1. Diversity of molecules reported to be endocytosed via non-clathrin mechanisms[a]

Soluble ligand	Membrane receptor	Rafts?	Cholesterol sensitive?	Itinerary in cell	Refs
Cholera toxin B subunit	GM1 (glycosphingolipid)	Yes	Yes	Endosomes, Golgi, ER	7,31
Shiga toxin B subunit	Gb3 (glycosphingolipid)	Yes	Yes	Endosomes, Golgi, ER	7,30
Verotoxin B subunit	Gb3	?	Yes	Endosomes, Golgi, ER	40
Diphtheria toxin	GPI-linked diphtheria toxin Receptor (an artificial chimera)	Yes	No	Acidic endosomal compartment	33
Murine leukaemia virus	Cationic amino acid transporter	?	Yes	?	49,50
SV40	MHC I	?	Yes	Caveosome, ER	5
Interleukin 2	IL-2 receptor	Yes	Yes	Late endosomes/lysosome (b subunit)	11
–	CD59	Yes	Yes	Golgi	7
–	GPI-linked GFP	Yes	Yes	Golgi	7
Factor VIIa	Tissue factor	?	?	?	51
Urokinase plasminogen activator	UPA receptor (GPI-linked)	Yes	?	?	34
	E-cadherin	Yes	Yes	?	52
Adenosine	A1 adenosine receptor and adenosine deaminase	Yes	Yes	?	53
Cytotoxic necrotizing factor 1	?	?	?	Acidic endosomal compartment	54
	N-Formyl peptide and C5a chemoattractant receptors	?	?	?	32
Adrenaline	Muscarinic adrinergic receptors	?	?	?	36
Dopamine	Dopamine D2 receptors	?	?	?	35
Angiotensin	Angiotensin II type 1A receptor	?	?	?	55
Interferon γ	Interferon γ receptor	?	?	?	56

[a]Viruses and bacterial toxin subunits are separated from non-pathogenic ligands.

low-density lipoprotein (LDL) receptor, are excluded from rafts[8,10]. Although stringent depletion of cholesterol from cell membranes is likely to have a wide range of effects beyond disruption of raft organization at the plasma membrane, some studies reveal specific conditions of cholesterol perturbation that inhibit non-clathrin pathways, without significant effects on clathrin-dependent uptake[6,7,11]. This hints at an important relationship between the properties and organization of cholesterol-sensitive raft microdomains on the plasma membrane and non-clathrin uptake pathways. However, the diversity of intracellular itineraries followed by markers for non-clathrin endocytosis is difficult to reconcile with their stable association with the same uniform microdomains. Thus, as discussed in detail later in this article, our increasing knowledge of non-clathrin endocytic pathways places some constraints on the raft model.

Potentially, clathrin-independent pathways could play vital roles in cell-surface recycling and remodeling, regulation of plasma membrane lipid composition, uptake of particular toxins and viruses and regulation of signal transduction. Much needs to be learned regarding the molecular and mechanistic operation of these pathways and their specific function(s) within cells. As discussed below, recent findings are beginning to clarify the machinery involved and the transport routes used.

Caveolar uptake

Caveolae are flask shaped, non-coated plasma membrane invaginations present in many cell types, but are especially abundant in endothelial cells, where they are implicated in transcytosis and endocytosis of blood components (see Fig. 1). Biochemically, caveolae are characterized by their association with a family of cholesterol-binding proteins called caveolins, which function to create and/or maintain these structures[12]. Consistent with this, expressing caveolin in cells that do not normally have caveolae is apparently sufficient to generate these structures[13]. Several dozen membrane receptors, signaling molecules and membrane transporters localize to caveolae. This has led to the idea that they might act as centers for signaling activity within cells[14], in addition to their role in endocytosis of specific molecules. Importantly,

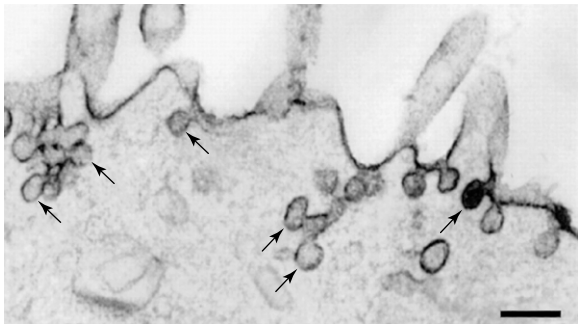

Fig. 1. Caveolae. Caveolae accumulating in a cell micro-injected with antibodies against dynamin 2. Image reproduced, with permission, from Ref. 15. Bar, 100 nm.

molecules found within caveolae are generally also present in lipid rafts[12].

The extent and mechanism by which caveolae mediate uptake of molecules within cells has been controversial. Potocytosis, involving direct diffusion of small molecules into the cytoplasm after association with caveolae, has been proposed as an alternative way caveolae mediate uptake of molecules without membrane internalization[12]. The finding that caveolae contain one key element of the machinery involved in vesicle budding, the GTPase dynamin[15,16], suggests that they also participate in membrane internalization. Consistent with this, *in vitro* assays have shown that dynamin and GTP mediate fission of caveolae from plasma membrane into free transport vesicles[15,16]. However, the extent of caveolar endocytosis in non-endothelial cells *in vivo* is not clear, and this might be an infrequent, possibly regulated, process[12].

Insight into the mechanism of caveolar internalization of cell-surface molecules has been obtained by visualizing the trafficking of cargo that internalizes through caveolae. It has long been known that the SV40 virus uses caveolae to gain entry into the cell, where ultimately it is delivered to the endoplasmic reticulum (ER)[17]. However, the nature of the intermediate trafficking steps involving SV40 and caveolin have been less clear. Recently, Pelkmans *et al.*[5] have employed a caveolin–GFP fusion protein, together with fluorescently conjugated SV40 virus, in experiments that employ imaging techniques to follow uptake of SV40 in living cells. Intriguingly, initial association of SV40 with the plasma membrane is a two-step process, with the virus first binding to laterally mobile major histocompatibility complex (MHC) class I antigens before becoming trapped in relatively stationary caveolae. Subsequent uptake of SV40 initially leads to delivery to intracellular organelles that are distinct from classical transferrin-labeled endosomes. The presence of caveolin in these organelles suggested the name 'caveosome'. SV40 then segregates from caveolin and is sorted out of caveosomes for delivery to the ER. Caveosomes are not continuous with the plasma membrane, and thus they provide the first example of a specific intracellular target organelle for caveolar endocytosis. One striking property of caveosomes, which emerges from the work of Pelkmans *et al.*[5], is their stability. In the absence of SV40, these organelles can persist without significant movement for several hours, in marked contrast to the dynamic nature of transferrin-labeled early endosomes. Whether membrane components, including caveolin, exchange between the plasma membrane and caveosomes over this time remains to be ascertained, and elucidation of the constitutive function of caveosomes is an exciting challenge for the future. The availability of the caveolin–GFP construct will greatly facilitate resolution of these issues.

Macropinocytosis

Macropinocytosis refers to the formation of large, irregular primary endocytic vesicles by the closure of lamellipodia generated primarily at ruffling membrane domains. Macropinosomes are dynamic structures, frequently moving inwards towards the centre of the cell, and persist for approximately 5–20 min (Ref. 18). The membrane components of macropinosomes are likely to be recycled back to the plasma membrane over a similar time-scale, potentially passing via other organelles within the cell, and macropinosomes must recruit specific machinery in order to do this. Although there is as yet no direct evidence that the composition of macropinosomes is different from that of the plasma membrane ruffles from which they are derived, ruffles themselves might have a different composition from the rest of the plasma membrane as there are reports that both specific phosphoinositides and lipid raft markers are enriched in ruffling membrane[19,20].

Recycling of membrane between the plasma membrane and endosomal compartments during macropinocytosis is most likely regulated by the small GTPase ARF6[21,22]. Overexpression of either mutant ARF6 locked in its GTP-bound form, or the EFA6 GDP–GTP exchange factor for ARF6, results in both ruffling and the accumulation of macropinosomes. These structures recruit a GFP construct based on the phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2]-binding pleckstrin-homology (PH) domain of phospholipase Cγ[23], and ARF6 activates phosphatidylinositol 4-phosphate 5-kinase at the plasma membrane[24]. Thus localized PtdIns(4,5)P_2 production is likely to be one important means by which the dynamics of macropinosomes are regulated. Consistent with this, constitutive macropinocytosis in oncogene-transformed fibroblasts requires constant PtdIns(4,5)P_2 turnover[25]. This raises the question of what effectors lie downstream of ARF6-mediated PtdIns(4,5)P_2 production. Components of the actin-based cytoskeleton are obvious candidates. Also, the finding that human spectrin SH3-domain-binding

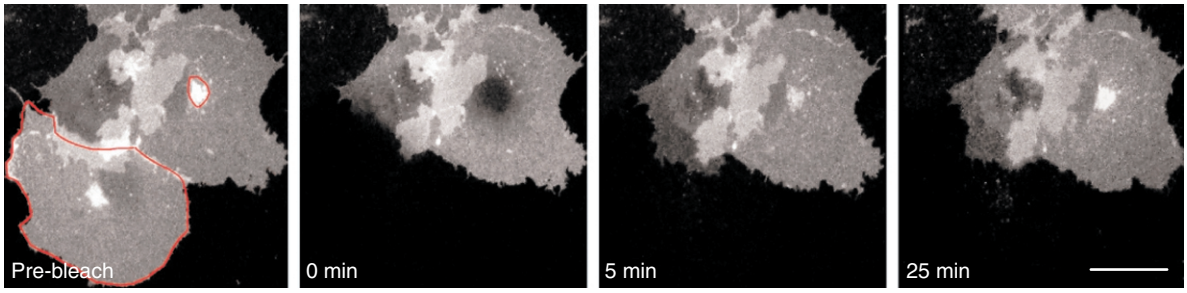

Fig. 2. Clathrin-independent exchange of a marker for lipid rafts between the plasma membrane and Golgi complex. Selective photobleaching was used to assay exchange of glycosylphosphatidylinositol linked to green-fluorescent protein (GPI–GFP) between the cell surface and Golgi pools in cycloheximide-treated COS-7 cells[7]. The areas enclosed by a red line in the pre-bleach image were photobleached. The times shown in the subsequent panels denote minutes post-bleach. The cell to the bottom left was completely photobleached as a control for absence of new protein synthesis. In the absence of such synthesis, the Golgi pool of GPI–GFP in the upper cell recovers, at the expense of plasma membrane fluorescence. This recovery is unaffected by epsin and eps15 mutants that disrupt clathrin coats[7].

protein 1, or Hssh3bp1, is recruited specifically to macropinosomes through binding to a spectrin-like ligand implies that the spectrin–ankyrin-based cytoskeleton provides an additional class of potential ARF6 effectors[26]. Moreover, Hssh3bp1 provides the first example of a protein found on macropinosomes but not on the ruffles from which they are derived.

Although the involvement of localized PtdIns(4,5)P_2 production in macropinocytosis, and a role for ARF6 in regulating this, appear well established, whether ARF6 acts in both the production and consumption of macropinosomes, whether the structures produced by artificial ARF6 activation correspond to the macropinosomes normally associated with ruffles, and the extent to which also ARF6 regulates other endocytic pathways, remain to be resolved.

An understanding of the function of ruffle-associated macropinocytosis is related to the wider question of the significance of ruffles themselves in activated or motile cells. This somewhat contentious point has been discussed at length elsewhere[27]. Macropinosomes might simply represent membrane internalized as a side-effect of the very pronounced membrane and cytoskeletal activity within the ruffle, or alternatively might represent an internal reservoir of ruffle-like membrane containing raft lipids and associated signaling molecules, the recycling of which could play a crucial role in generating inhomogeneity in the plasma membrane during cell activation and motility. The finding that ARF6 activation is sufficient to influence both membrane trafficking and cortical actin dynamics argues in favor of the latter possibility[21–23].

Constitutive clathrin-independent endocytosis

Macropinosomes are typically associated with ruffling membrane and not often found in resting, unstimulated cells. However, even in unstimulated cells, there are likely to be constitutive clathrin-independent endocytic pathways, as described in several recent papers[6,7,11]. Lamaze *et al.*[11] found that endocytosis of interleukin 2 (IL-2) receptors proceeded normally in cells where clathrin function was disrupted by use of eps15 mutants. Eps15 binds directly to the plasma membrane adaptor AP-2 as well as to several other proteins of the clathrin-associated endocytic machinery, and overexpression of individual domains from eps15 disrupts the network of protein–protein interactions required for clathrin-mediated uptake[3]. Ultrastructural and biochemical experiments showed that clathrin-independent endocytosis of IL-2 receptors occurs constitutively in lymphocytes, which are devoid of caveolae and caveolin, and is coupled to partitioning of IL-2 receptors into lipid rafts. The itinerary of IL-2 receptors after internalization is not well characterized, although ultimately they are delivered to lysosomes[11].

Puri *et al.*[6] used the same eps15 mutant in human skin fibroblasts to show that fluorescent analogs of lactosylceramide and globoside were internalized almost exclusively by a clathrin-independent mechanism, while an analog of sphingomyelin was taken up approximately equally by clathrin-dependent and -independent pathways. All three lipid analogs accumulated in the Golgi apparatus. The idea of a constitutive clathrin-independent pathway from the plasma membrane to the Golgi apparatus is further supported by previous studies on the uptake of exogenous lipids[28,29] and by another recent paper[7]. Several markers for lipid rafts, including both GPI-anchored proteins and lipid-binding subunits of bacterial toxins (see below), were shown to be endocytosed to the Golgi apparatus independently of clathrin[7]. Again, mutant eps15 was used, together with a dominant-negative mutant of epsin that has a similar inhibitory effect on clathrin function. The use of selective photobleaching techniques in living cells allowed derivation of the rate constants describing exchange of a GFP–GPI anchor between plasma membrane and Golgi pools (Fig. 2). Uptake from the plasma membrane to the Golgi took place at about 0.5% min^{-1}. This is less efficient than clathrin-mediated uptake of, for example, the transferrin receptor, into early endosomes, which occurs at a least 10% min^{-1} (Ref. 2). However, even uptake at 0.5% min^{-1} would

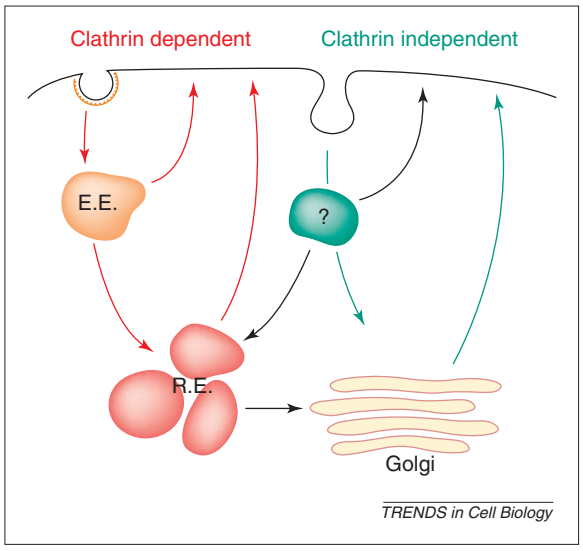

Fig. 3. Two independent endocytic cycles. The red arrows outline the itinerary followed by a classical endocytic marker, such as transferrin, via clathrin-coated pits, early endosomes (E.E.) and recycling endosomes (R.E.). The green arrows outline a separate, more cholesterol-sensitive cycle followed by markers for lipid rafts, such as some GPI-linked proteins, and plasma membrane lipids[6,7]. The question mark reflects the fact that intermediate endocytic compartments, or indeed the internalization mechanism, in the clathrin-independent cycle have not been characterized. Caveosomes[5] provide candidate structures, as discussed in detail in the text. Black arrows denote potential points of intersection between the two cycles, which can nonetheless function independently[6,7,30,31].

allow all of the GPI-linked GFP, and by inference many other markers for lipid rafts in the plasma membrane, to cycle via the Golgi apparatus once every 2–3 hours[7].

One central conclusion emerging from the papers discussed above[6,7] is that there is a constitutive, cholesterol-sensitive pathway carrying plasma membrane lipids and markers for lipid rafts from the plasma membrane to the Golgi apparatus (Fig. 3). However, several questions need to be resolved. The magnitude of the flux of endogenous proteins and lipids between the plasma membrane and the Golgi, which is obviously an issue relevant to potential functions of the pathway, remains difficult to investigate directly. The intermediate steps in the pathway are also unclear. Either raft markers could, after uptake through a non-clathrin mechanism, converge with the classical early and recycling endosome system and then be sorted out for delivery to the Golgi (Ref. 30) or they could bypass this membrane system altogether[7] (see Fig. 3). In the latter case, caveolar uptake and delivery to caveosomes could be involved, and one major question for the future is whether raft markers bound for the Golgi pass through these organelles.

Caveolin-dependent transport via caveolae and caveosomes is unlikely to provide the only constitutive clathrin-independent endocytic pathway. Constitutive clathrin-independent endocytosis of both IL-2 receptors and cholera toxin B subunit occurs in cells lacking caveolin[11,31]. Moreover, there are several examples of proteins internalized independently of dynamin, and dynamin is thought to be required for both caveolar and clathrin-mediated uptake[32–36]. How such clathrin-independent, caveolin-independent uptake might occur is not clear, but it is tempting to speculate that ARF6, which is found on transferrin-negative endocytic membranes in HeLa cells, might well be involved[22]. In that case, the initial internalization step might be mechanistically related to the formation of macropinosomes.

Bacterial toxins as markers for clathrin-independent endocytosis

Several bacterial toxins enter the cell by binding to cell-surface receptors, being endocytosed, and ultimately translocating into the cytosol from an intracellular compartment. A number of these toxins are taken up independently of clathrin and hence provide valuable markers[37]. However, the diversity of endocytic pathways and potential for simultaneous uptake through multiple routes frequently makes interpreting the literature complex. A case in point is provided by perhaps the best-studied bacterial toxin, cholera toxin[38]. Intact cholera toxin has two subunits, the A subunit being responsible for toxic ADP-ribosylating activity, whereas the B subunit mediates uptake into the cell by binding to GM1, a glycosphingolipid found both in caveolae and elsewhere on the plasma membrane, within lipid rafts. The appealingly simple assumption that uptake of the cholera toxin B subunit directly and exclusively corresponds to budding of caveolae from the plasma membrane is almost certainly wrong. Cholera toxin B subunit and GM1 are also detected in clathrin-coated pits[39]. Pharmacological studies on the uptake of cholera toxin show that agents that perturb clathrin function reduce the total amount of toxin internalized into cells[31,40,41]. This approach also reveals a crucial difference between toxin internalization, which is in part clathrin dependent, and toxin activity, which is not[31]. For cholera toxin to be toxic, it must first reach the Golgi apparatus[38], so this implies that, although cholera toxin can be taken up through multiple pathways, only non-clathrin pathways lead to delivery to the Golgi. The use of specific inhibitors of clathrin-mediated internalization has allowed direct confirmation of this conclusion. Thus, mutants of epsin and eps15 cause a partial decrease in the total amount of cholera toxin B subunit taken up into the cell, with no measurable effect on delivery to the Golgi apparatus[7].

The literature on cholera toxin B subunit supports the idea that delivery of raft markers to transferrin-containing early or recycling endosomes is not coupled to delivery to the Golgi (Fig. 3). This is consistent with data on the endocytic distribution of the folate receptor, a GPI-anchored protein also found in lipid rafts, which accumulates in recycling endosomes without subsequent delivery to the

Golgi[42]. However, another bacterial toxin subunit, Shiga toxin B subunit, is thought to travel from recycling endosomes to the Golgi apparatus[30,43]. Moreover, a fluorescent analog of sphingomyelin, which, like cholera toxin B subunit, can be taken up by both clathrin-dependent and -independent mechanisms, can be delivered to the Golgi through either of these two alternative uptake pathways[6]. Thus, although there is now good evidence from several sources for a clathrin-independent pathway from the plasma membrane to the Golgi apparatus[6,7,31], the extent of cross-talk between this pathway and conventional early and recycling endosomes needs to be investigated in more detail.

Non-clathrin endocytosis and lipid rafts

Given the ubiquity and diversity of clathrin-independent uptake pathways, an important conclusion must be that the coated-vesicle paradigm is far from the only way in which cells have solved the problem of transporting material between different organelles while maintaining the specific membrane composition of each individual organelle. Partitioning into putative microdomains on the plasma membrane, or rafts, offers an attractive explanation for how sorting into clathrin-independent uptake pathways might occur. The observations that typical markers for clathrin-mediated endocytosis such as the transferrin receptor and the LDL receptor are excluded from rafts[8], and that cholesterol depletion can, under carefully controlled conditions, perturb partitioning into rafts and simultaneously block non-clathrin uptake without perturbing endocytosis of transferrin[6,7,31], add further weight to this idea. However, as discussed below, a simple model for lipid rafts as stable, uniform structures, does not fit well with the data.

Different markers for rafts accumulate in different endocytic compartments. Even different GPI-anchored proteins have different uptake pathways, with some cycling between the cell surface and the Golgi apparatus, whereas others cycle via recycling endosomes[7,42]. Moreover, cholera toxin B subunit has an endocytic distribution that is different again, entering both recycling endosomes and the Golgi. Clearly, the sorting of these molecules cannot be principally determined by co-clustering in the same stable microdomain. Direct evidence that raft association is not the only factor affecting the sorting of raft markers is provided by experiments showing that the β subunit of the IL-2 receptor, which is internalized in a raft-dependent fashion, is delivered to late endosomes and lysosomes for degradation, and this requires ubiquitination of a specific sorting signal[44]. Markers for lipid rafts are frequently found within caveolae[10], but, as discussed already, the finding that such markers are taken up efficiently in the absence of caveolae undermines the simple model where uptake of raft markers solely corresponds to budding of caveolae from the plasma membrane. A functional distinction between caveolae and lipid rafts is suggested by both sub-fractionation and confocal microscopy experiments showing that heterotrimeric G proteins (Gi, Gq, Gs and Gβγ) are differentially targeted to caveolae and to discrete cell-surface microdomains, or rafts[45]. We have argued that raft markers can be taken up either through caveolae, or through another, currently uncharacterized, clathrin-independent mechanism. These observations raise the possibility that there are different types of raft, sorted differently by the cell. Alternatively, in the absence of detergent, lipid rafts might be rather less stable entities than is currently thought, allowing for independent sorting of raft markers away from each other.

'...a cholesterol-sensitive pathway carries markers for lipid rafts from the plasma membrane to the Golgi apparatus...'

There are clearly many unanswered questions about both the nature of lipid rafts themselves and the non-clathrin pathways that allow uptake of markers for these microdomains. However, the observation that several markers for rafts do indeed follow constitutive non-clathrin endocytic pathways provides hints as to what the functional importance of these pathways might be. Rafts are thought to play a role in sorting at the *trans*-Golgi network of both polarized and non-polarized cells, and raft lipids may well have a more general role in Golgi organization[46–48]. It is possible that recycling of raft markers from the plasma membrane to the Golgi via a non-clathrin endocytic pathway plays an important role in these events. A related potential function of clathrin-independent recycling of markers for rafts is in the selective endocytosis of particular cell-surface components during generation of polarized cell-surface domains. Differential endocytosis of raft markers could well help to generate and maintain the in-homogeneous distribution of such markers in the plasma membrane that is observed both in epithelia and in migrating cells[8,19].

Concluding remarks

In this article, we have highlighted the diversity of endocytic pathways found within mammalian cells, and argued that clathrin-independent endocytosis is likely to play an important role in membrane dynamics and in remodeling of the plasma membrane in response to external stimuli. The most obvious challenges for the future are to provide a better description of the molecular machinery that mediates these pathways and to better characterize their spatial and temporal regulation.

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